SUPPLEMENTAL MATERIAL

Detailed Methods

Family Recruitment and Characterization

The study protocol was approved by the Institutional Review Board of the University of Texas Health Science Center at Houston. Families with two or more members affected with TAAD were enrolled into the study. Phenotypic characterization of vascular diseases, including TAAD, ICAs and AAAs, was previously described [1]. Blood or saliva samples were obtained from affected individuals and family members. Medical records, including imaging studies of the aorta and cerebral vessels, surgical reports, hospital records, and physicians' notes were reviewed. Phenotypic features beyond the vascular system were assessed in eight *SMAD3* mutation carriers by clinical geneticists. *SMAD3* mutation carriers were interviewed concerning joint pain and complaints, and the medical records were reviewed for diagnosis of osteoarthritis. The ethnicity of the 181 FTAAD probands was 86% European American, 5% African American, 1% Asian, 4% Hispanic and 3% other ethnicity. *SMAD3* variants were only identified in European Americans.

Targeted capture and massive parallel sequencing

Genomic DNA was extracted from peripheral blood lymphocytes using standard protocols. Five micrograms of DNA from two affected individuals in family TAA549 (1/16 coefficient of relatedness) were used for construction of the shotgun sequencing library as described previously using adaptors for paired-end sequencing[2]. Libraries underwent exome capture using the ~34 Mb target from Roche/Nimblegen SeqCap EZ v2.0. Briefly, 1 µg of shotgun library was hybridized to biotinylated capture probes for 72 hours, enriched fragments subsequently recovered via streptavidin beads, and PCR amplified. Enriched libraries were then sequenced on an Illumina GAIIx with paired end 76 base reads using manufacturer protocols. Each sample generated ~50M unique reads mapping to the exome target and nearby flanking regions, with >95% of the exome positions having a depth of >8x coverage, and an average coverage of 71x overall.

Read mapping and variant analysis

Each sample was processed from real-time base-calls on the GAIIx instrument (RTA 1.7 software [Bustard], converted to qseq.txt files, and aligned to a human reference (hg19) using BWA (Burrows-Wheeler Aligner)[3]. Read-pairs not mapping within ±2 standard deviations of the average library size (~125 ± 15 bp for exomes) were removed. Data was processed using the Genome Analysis ToolKit[4](GATK refv1.2905). All aligned read data was subjected to "duplicate removal", i.e. the removal of reads with duplicate start positions, indel realignment (GATK IndelRealigner) and base qualities recalibration (GATK TableRecalibration).

Variant detection and genotyping are performed using the UnifiedGenotyper (UG) tool from GATK. Variant data for each sample was formatted (variant call format [VCF]) as "raw" calls for all samples, and sites flagged using the filtration walker (GATK) to mark sites that are of lower quality/false positives (e.g. low quality scores ($\leq$50), allelic imbalance ($\geq$0.75), long homopolymer runs (>3), and/or low quality by depth (QD<5)). Each sample generated an average of 27,280 total variants, with a final pass filter set of 24,282. Only variant sites passing all GATK filters (ie. pass) were analyzed.

Annotation of variants was performed using the SeattleSeq server (http://gvs.gs.washington.edu/SeattleSeqAnnotation/). The identified variants were then filtered against exome data from 21 non-affected control individuals for indel and SNV calls to identify novel non-synonymous and splice acceptor and donor site variant that was present as heterozygous genotype in both individuals. These variants were considered as candidate mutations.

Confirmation Sequencing and Linkage Analysis

Bidirectional DNA sequencing of candidate variants were done using primers designed 60-120 bp from the variant. PCR amplifications were carried out using HotStar Taq™ DNA polymerase (Qiagen Inc.Valencia, CA). PCR products were treated with EXOSAP-IT (Affymetrix, Inc. OH) to digest the primers and followed with sequencing PCR using the BigDye™ sequencing reaction mix (Applied Biosystems, CA). The sequencing PCR products were purified using the BigDye XTerminator kit (Applied Biosystems, CA) and then loaded on an ABI3730xl sequencing instrument using the Rapid36 run module. DNA sequencing results were analyzed using the Mutation Surveyor software (SoftGenetics, PA). *SMAD3* sequencing of all exons and flanking introns were carried out using DNA from 181 probands with FTAAD and *SMAD3* mutations were reported based on the RefSeq codes NM_005902.3 (*SMAD3* mRNA*)* and NP_005893.1 (SMAD3 protein). The *SMAD3* rare variants identified in family TAA549 and 4 FTAAD probands were not present in approximately 2300 exomes from the Exome Sequencing Project (approximately two-thirds European descent and one-third African descent).[5] The mutational status of family members, who carry the mutation but are unaffected and not essential in demonstrating segregation of the mutation with the disease phenotype are not reported in the pedigrees.

Two-point linkage analysis with candidate variant status was performed in the families with *SMAD3* mutations. An affected-only analysis was done with unknown and unaffected individuals both designated as unknown as far as vascular disease status in the analysis. The disease-allele frequency were defined as previously described and 0.001 was the minor allele frequency of the candidate variants.[6] LOD scores were calculated with MLINK program of the computer software FASTLINK, version 3.P.[7]

Supplemental Table

Online Table I. Assessment of Marfan and Loey-Dietz features by a geneticist in eight patients with *SMAD3* mutation (See Figure 1)

Clinical feature	# of patients/total patients assessed
Skeletal	
Arachnodactyly	0/8
Pectus deformity	1/8 (minimal pectus carinatum)
Scoliosis	1/8 (minimal scoliosis)
Camptodactyly	1/8
Joint laxity	4/8
Protrusio acetabulae	unknown
Pes planus	3/8
Craniofacial	
Long face	1/8
Hypertelorism	0/8
Flat supraorbital ridges	0/8
Malar hypoplasia	3/8
Cleft palate	0/8
High arched palate	0/8
Broad uvula	1/8
Bifid uvula	1/8
Dental malocclusion	0/8
Skin/integument	
Velvety skin	0/8
Translucent skin	2/8
Easy bruising	2/8
Atrophic scars	1/8
Striae	0/8
Hernia	4/8
Dural ectasia	1/8

Supplemental References

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